

(13)

18th PLANT DEVELOPMENT WORKSHOP

Saturday, November 1, 1986

Department of Botany, University of Guelph, Guelph, Ontario

PRELIMINARY SCHEDULE

9:00- 9:30	Registration, coffee
9:30-12:00	Contributed papers
12:00-2:00	Lunch and poster session
2:00-4:30	Contributed papers
4:30-5:00	Reception

PRESENTATIONS

Please submit an abstract typed in a box 17 cm. wide by 10 cm. deep. Give title, author, address and the text. Please indicate clearly if you wish this to be a talk or a poster presentation. Talks will be limited to 20 minutes including questions and discussion. **Deadline for submission of abstracts.....Friday October 17th.**

SEND ABSTRACTS TO:

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Guelph, Ont. N1G 2W1

Telephone: (519) 824-4120 ext. 2745 (U. Posluszny) or 3278 (L. Peterson)

INFORMATION REQUESTED:

Please provide the following information by **Friday October 17th:**

The number that will attend from your lab_____

The number of talks and/or posters you will present_____

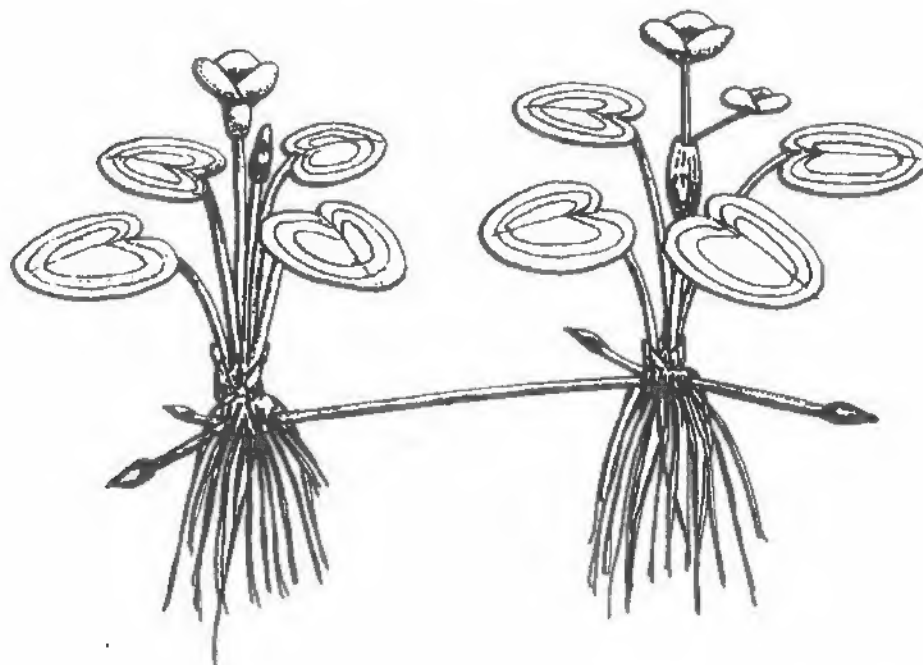
Any requirements for accommodation_____

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Saturday, November 1, 1986

Department of Botany, University of Guelph, Guelph, Ontario

PROGRAMME & ABSTRACTS



Hydrocharis morsus-ranae

drawn by R. Scribailo

NOTE: Registration, talks and posters will all take place in the University Centre (see enclosed map...Building No. 158), on the 4th floor, rooms 441 and 442.

LUNCH: There are light lunches available in the cafeterias on the first floor of the University Centre or at The Mountain Pizza in the residence next to parking lot P8.

PARKING: The best places to park are in lots P1, P4 and P42.

RECEPTION: There will be a wine and cheese reception at the Faculty Club on the 5th floor of the University Centre. We will be collecting a small fee to offset the cost of beer and wine. Please purchase a ticket at registration if you intend to go to the reception.

18th Plant Development Workshop
Program

8:30 Registration and Coffee

Morning Session

9:00 Duchesne, L. C., R. L. Peterson & B. E. Ellis -- The ectomycorrhizal fungus *Paxillus involutus* induces resistance to *Fusarium oxysporum* in *Pinus resinosa*.

9:20 Massicotte, H. B., C. A. Ackerley & R. L. Peterson -- The root-fungus interface as an indicator of symbiont interaction in ectomycorrhizae.

9:40 Bernards, M. -- Suspension cultures of tomato (cv. Craigella) maintain resistance to *Verticillium albo-atrum* *in vitro*.

10:00 Newsted, W. J. & N. P. A. Huner -- The immunological relatedness and localization of major developmental proteins in four psychrophilic fungi.

Coffee Break

10:40 Penrose, D. M. & B. R. Glick -- Tissue-specific expression of phosphoenolpyruvate carboxylase in sorghum.

11:00 Glick, B. R., D. M. Penrose, T. R. Harrington & N. W. Lem -- *Anabaena variabilis* phosphoenolpyruvate carboxylase: Enzyme kinetics and gene isolation.

11:20 Deslauriers, C., A. D. Powell and K. P. Pauls -- Flow cytometric characterization of an embryogenic *Brassica napus* microspore culture.

11:40 Moore, M. I. -- The membrane-enclosed, removable sphere in the nucellar cavity of *Pinus banksiana* Lamb.

12:00 LUNCH AND POSTER SESSION

Afternoon Session

- 1:40 Eberl, D. F. & A. J. Hilliker -- Genetic analysis of embryonic development.
- 2:00 Mellerowicz, E. & R. T. Riding -- Nuclear DNA status of young and old cambia of balsam fir and its changes during winter dormancy.
- 2:20 Armstrong, S. W. -- Comparisons between mitotic cells in callus tissue and secondary roots of *Cocos nucifera* L.: Analysis of cell and nuclear area, and RNA and protein content.
- 2:40 Gao, J-g., & R. A. Fletcher -- Cytological and morphological changes in wheat leaves induced by triazoles.
- 3:00 Mackay, C. E., T. Senaratna, B. D. McKersie & R. A. Fletcher. -- Protection of wheat plants from ozone injury by the triazole S-3307.

Break

- 3:30 Stobbs, L. W. & J. G. Van Schagen -- Occurrence and characterization of a turnip mosaic virus isolate infecting *Allaria petiolata* (M. Bieb) in Ontario, Canada.
- 3:50 Stobbs, L. W. & D. Barker -- Rapid sample analysis with a simplified ELISA.
- 4:10 Stobbs, L. W., J. W. Potter, R. Killins, & J. G. van Schagen -- Influence of grapevine understock in infection of 'Dechaunac' scion by tomato ringspot virus.
- 4:30 Stobbs, L. W. & J. G. Van Schagen -- Effect of shoot thinning on the production of graftable wood from four grape rootstocks.

Wine and Cheese Reception

POSTER TITLES:

Bommineni, V. R., R. I. Greyson, D. B. Walden & B. G. Atkinson --
Differentiation of polypeptides with the development of corn (*Zea mays* L.) inflorescences.

Gerrath, J. M. & U. Posluszny -- Early ontogeny of the
tendrill/inflorescence in *Vitis riparia*.

Krol, M., N. Huner, J. P. Williams & E. Maissan -- Chloroplast development
and low temperature affects the fatty acid composition of
phosphatidylglycerol and LHCII assembly.

Laroche, A. & W. G. Hopkins -- Larger and more active polysomes are
assembled at low temperature.

Pareddy, D. R. -- Analysis of variability in plants produced with pollen
from *in vitro* cultured tassels of *Zea mays*.

Rauser, W. E. & C. A. Ackerley -- Localization of cadmium in granules
within differentiating and mature root cells.

ABSTRACTS

The ectomycorrhizal fungus Paxillus involutus induces resistance to Fusarium oxysporum in Pinus resinosa.
by Luc C. Duchesne, R.L. Peterson and B.E. Ellis. Department of Botany, University of Guelph, Guelph.

Survival of Pinus resinosa Ait. seedlings after exposure to the root pathogen Fusarium oxysporum f.sp. pini shows a 50% increase when the seedlings are also inoculated with the ectomycorrhizal fungus Paxillus involutus Fr. Bioassay of the rhizosphere and of root extracts from seedlings inoculated with P. involutus indicates that induced resistance to F. oxysporum is associated with antimicrobial compounds in the rhizosphere. Culturing P. involutus on Modified Melin Norkrans medium alone or with root exudates of P. resinosa shows that antibiotic production by P. involutus is induced by pine root exudates. Our results also suggest that disease suppression by P. involutus is mostly associated with antibiotic production by this fungus. These findings may be significant in terms of seedling survival in the field and may be important in afforestation programs in Canada.

The root-fungus interface as an indicator of symbiont interaction in ectomycorrhizae

H.B. MASSICOTTE*, C.A. ACKERLEY and R.L. PETERSON
Department of Botany, University of Guelph, Guelph, Ontario

Seedlings of Alnus crispa (Ait.) Pursh, Alnus rubra Bong., Eucalyptus pilularis Sm. and Betula alleghaniensis Britt. were grown in plastic pouches and subsequently inoculated with Alpova diplophloeus (Zeller and Dodge) Trappe and Smith (2 different strains), Pisolithus tinctorius (Pers.) Coker & Couch and Laccaria bicolor (R. Mre) Orton respectively to form ectomycorrhizae in situ. Alnus seedlings were inoculated with Frankia prior to inoculation with the mycosymbiont. The interface established between A. crispa and A. diplophloeus was complex involving wall ingrowth formation in root epidermal cells and wall branchings in Hartig net hyphae. A. rubra - A. diplophloeus ectomycorrhizae had an interface lacking epidermal cell wall ingrowths but with labyrinthine wall branching of Hartig net hyphae. The interface between E. pilularis - P. tinctorius consisted of branching Hartig net hyphae between radially-enlarged epidermal cells lacking wall ingrowths. Ectomycorrhizae between B. alleghaniensis - L. bicolor developed unique interfaces with the radial enlargement of epidermal cells near the apical meristem which synthesized dense vacuolar deposits. Very fine branchings occurred in Hartig net hyphae.

Suspension Cultures of Tomato (cv. Craigella) Maintain Resistance to Verticillium albo-atrum in vitro

Mark Bernards, University of Guelph Dept. Chemistry/Biochemistry

In vitro co-cultivation of Verticillium albo-atrum with cultures of tomato cv. Craigella carrying the Ve gene for resistance to this fungal pathogen leads to a reduction in mycelial growth relative to that observed when the fungus is co-cultivated with a near isoline of Craigella lacking the Ve gene. After three days incubation, little mycelial growth is observed on the resistant isoline, while growth on the susceptible isoline is almost equal to that of control fungal cultures. Spore counts and chemical analysis of amino sugars are being used to quantify this observed differential in fungal growth.

The immunological relatedness and localization of major developmental proteins in four psychrophilic fungi. W.J. Newsted and N.P.A. Huner, Department of Plant Sciences, University of Western Ontario, London, Ontario, Canada. N6A 5B7.

Four psychrophilic fungi, Myriosclerotinia borealis (W51), Coprinus psychromorbidus (LRS131), Typhula idahoensis (W21) and Typhula incarnata (W29) were grown in darkness at 5°C on a defined agar medium until vegetative hyphae, sclerotial initials and mature sclerotia were formed. Polypeptide complements of these structures were compared by 1D and 2D SDS-PAGE. Results indicated the presence of major low molecular mass polypeptides ranging from 12-30kD in the sclerotia of all four fungi. However, the number and molecular mass of the major sclerotial polypeptides varied from species to species. In addition, polypeptides of similar molecular mass were generally present in the sclerotial initials but were either absent or present in very low quantities in the vegetative hyphae. Ouchterlony immunodiffusion, using polyclonal antibodies raised to the purified major sclerotial polypeptides, indicated antigenic similarity among the major sclerotial polypeptides. This may be suggestive of a common role or function for the major sclerotial polypeptides. Thick sections of mature sclerotia stained for proteins revealed the presence of copious protein bodies in the cytoplasm of sclerotial cells of LRS131, W21 and W29. With immunofluorescence microscopy using Protein A-FITC as the fluorescent label, we established, for the first time, that the major sclerotial polypeptides of LRS131, W21, W29 were sequestered in these protein bodies.

Tissue-Specific Expression of Phosphoenolpyruvate Carboxylase in Sorghum. Donna M. Penrose and Bernard R. Glick. Department of Biology, University of Waterloo.

Phosphoenolpyruvate carboxylase (PEP-C) purified from young leaves of the C4 plant Sorghum bicolor has a native molecular weight of approximately 415,000 daltons, a monomeric molecular weight of approximately 100,500 daltons and a specific activity of 30 units/mg protein. Rabbit antibodies produced against the dissociated form of the enzyme were used to develop an ELISA for PEP-C in sorghum. The 10-fold difference in ELISA response observed between crude and partially purified PEP-C is consistent with the 10-fold purification of the enzyme suggested by densitometric traces and the 12-fold purification indicated by enzyme assays. Rabbit antibodies were used to monitor the levels of PEP-C protein during plant development. In greening leaves, a rapid increase in PEP-C protein (detected immunologically) was paralleled by an increase in PEP-C specific activity. In shoots, PEP-C protein and specific activity were present in moderate and relatively constant levels. In roots, PEP-C protein attained a moderately high level peaking at approximately 18 days after the seeds were planted while the specific activity of PEP-C in roots showed a somewhat smaller peak 24 days after the seeds were planted.

Anabaena variabilis Phosphoenolpyruvate Carboxylase: Enzyme Kinetics and Gene Isolation. Bernard R. Glick, Donna M. Penrose, Tina R. Harrington and Nora W. Lem. Department of Biology, University of Waterloo.

The enzyme phosphoenolpyruvate carboxylase (PEP-C) from the filamentous diazotrophic cyanobacterium A. variabilis was partially purified and kinetically characterized. The purified enzyme displayed kinetic behaviour (especially the response of the enzyme to a variety of potential allosteric effectors) which more closely resembled the response of PEP-C from C4 plants than from C3 plants to these effectors. This result raises the possibility that A. variabilis could have a functional C4 pathway without the anatomical features normally associated with this pathway in higher plants.

Purified A. variabilis chromosomal DNA partially digested with restriction endonuclease Sau3A was ligated into the BamHI site of plasmid pBR322. The resultant gene library, first established in E. coli HB101, was used to transform E. coli 342-167, a mutant with a decreased level of PEP-C activity. A transformant of E. coli 342-167 that grew on minimal media in the absence of glutamate was isolated and its plasmid (pTRH1) was shown to encode the A. variabilis PEP-C. E. coli HB101 cells transformed with this plasmid have approximately 50 times the normal amount of PEP-C activity.

Flow Cytometric Characterization of an Embryogenic Brassica napus microspore culture

C. Deslauriers, A.D. Powell and K.P. Pauls, Crop Science Dept., University of Guelph, Guelph, Ontario M1G 2W1

Immature microspore cells for B. napus can be redirected from pollen development toward haploid embryo development by culturing them in a medium containing mineral salts, vitamins, 13% sucrose, 0.05 mg/l BA and 0.5 mg/l NAA. Flow cytometry was used to measure the fluorescence and cell size of microspores stained with the vital dye fluorescein diacetate, during the first 7 days of culture. The results indicated that only 40% of the microspores were viable immediately after isolation and that viability declined to 3% by day 7. The size of the viable cells doubled within 1 day and continued to increase on subsequent days but at a slower rate. Cell divisions were apparent by day 3.

Flow cytometry was used to separate the live from the dead microspores at day three. The sorted cells continued to develop and produce embryos. This methodology will be used in the future to produce a uniform microspore culture for studies of the biochemical and molecular processes which accompany embryogenesis.

THE MEMBRANE-ENCLOSED, REMOVABLE SPHERE IN THE NUCELLAR CAVITY OF Pinus banksiana Lamb.

Mary I. Moore. Box 159, Deep River, Ont. K0J 1P0.

A glutaraldehyde- glycol methacrylate study of Pinus banksiana during the free nuclear - coenocytic ring period raises several questions. In fixed material, where is the division between nucellus and the membrane-covered central sphere that is so obvious in fresh material? What is the possible effect of fixative on the ring and associated tapetum? Free nuclei are sometimes raised above cytoplasm of the ring. Is this an artifact?

Nuclear DNA status of young and old cambia of balsam fir and its changes during winter dormancy.

E. Mellerowicz, R.T. Riding, University of New Brunswick, Bag Service No. 45111, Fredericton, N.B., Canada E3B 6E1

Feulgen-DNA content of old cambial cells was higher than young cambial cells. In both types of cambia there was an increase in nuclear DNA content during early winter dormancy. In young cambia, nuclei accumulated in the G1 stage during rest. DNA content then increased to the 3C level during the rest-quiescence transition (=emergence from deep dormancy of the cambium). In older cambia, the DNA increase occurred soon after the onset of dormancy. It is hypothesized that the old cambia may either lack rest or have rest of very short duration. The observed Feulgen-DNA increase probably represents metabolic DNA, but there is also a possibility of increased Feulgen detection due to structural changes in the chromosomes. This will be studied using a radioactive DNA precursor.

This work was supported by the grant from the Canadian Forestry Service to RTR.

COMPARISONS BETWEEN MITOTIC CELLS IN CALLUS TISSUE AND SECONDARY ROOTS OF COCOS NUCIFERA L.: ANALYSIS OF CELL AND NUCLEAR AREA, AND RNA AND PROTEIN CONTENT.
S.W. Armstrong, Department of Biology, McMaster University, Hamilton.

The relationships between cell area, nuclear area and cellular-RNA and -protein content were determined for mitotic cells in several callus lines and compared to the corresponding relationships for cells in the secondary root meristems of Dwarf, Hybrid and Tall varieties of Cocos nucifera. Mitotic cells in callus tissue showed increases in cell size and cellular-RNA and -protein content compared with mitotic cells from Dwarf and Hybrid root tips. However, there was a disproportionate increase in cell size as compared to either RNA or protein changes and probit analysis revealed that these three parameters were less tightly coupled in callus cells than in root tip cells.

In contrast, nuclear area for prophase cells in callus tissue was very similar to that in prophase cells from roots of the variety from which the callus line was established. Overall, our results suggest that nuclear and cellular parameters are free to change independently of each other when cells are transferred to culture media. Furthermore, the failure to increase RNA and protein contents by amounts equivalent to changes in cell size may have detrimental effects on cell cycle progression in callus cells.

CYTOLOGICAL AND MORPHOLOGICAL CHANGES IN WHEAT LEAVES INDUCED BY TRIAZOLES.
Jian-guo Gao and R.A. Fletcher, Department of Environmental Biology, University of Guelph, Ontario N1G 2W1.

Light and electron microscopy studies showed that the triazoles, triadimefon and S-3307, had multiple effects on the morphology and cytology of wheat leaves. Seeds were treated with triadimefon at 0.1 (low) and 1.0 (high) and S-3307 at 0.001 (low) and 0.01 (high) g a.i./kg of seed. Both triazoles reduced the length, but increased the width and thickness of leaves. Mesophyll cells of treated leaves were thicker than controls and there were more layers of cells around the median and lateral vascular bundles of leaves treated with high concentration of S-3307. The length of epidermal cells was reduced and the width increased by both triazoles; leaf thickness was increased by high concentrations of triadimefon only. S-3307 increased the number of vascular bundles, whereas triadimefon had no effect on numbers but at high concentration increased the vascular bundle diameter. Both concentrations of S-3307 reduced the length of trichomes. Both triazoles increased chloroplast size, with the length both along the long and short axes being greater than in the controls. In general, the anatomical changes induced by triadimefon and S-3307 in wheat leaves were similar. The effects on stomata were, however, very different. Compared to the control, the stomata in the triadimefon treated leaves were constricted and sunken whereas in S-3307 the subsidiary cells were wider. The effects of the triazoles observed in the present study may in part account for the several plant growth regulatory activities reported earlier including growth retardation, stimulation of chlorophyll synthesis and protection against injury from water stress.

PROTECTION OF WHEAT PLANTS FROM OZONE INJURY BY THE TRIAZOLE S-3307. C. E. Mackay, T. Senaratna, B. D. McKersie and R. A. Fletcher Department of Environmental Biology (C.E.M, R.A.F) and Department of Crop Science (T.S., B.D.M), University of Guelph, Guelph, Ontario, N1G 2W1.

Seven day old wheat plants acutely exposed to ozone showed typical symptoms of ozone injury, ie. chlorosis, necrotic lesions, increased solute leakage and altered chlorophyll fluorescence. The phytotoxic symptoms were prevented in plants seed-treated with the triazole S-3307. Ozone treatment also induced a decline in phospholipid content and a concomitant increase in the levels of free fatty acids. Furthermore, there was no evidence of fatty acid peroxidation in that there was no significant decrease in fatty acid unsaturation with ozone fumigation. This supports the hypothesis that the major mechanism of ozone toxicity is mediated through a free radical cleavage of the ester linkages between the glycerol moiety and the fatty acids tails of phospholipids. The cleavage of phospholipids was not evident in the plants pretreated with S-3307 and it is suggested that this was due to increases in lipid soluble antioxidants found in the triazole treated plants.